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## Towards personalized therapy for metastatic prostate cancer: technical validation of [18F]fluoromethylcholine

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## CHAPTER 7

# GENERAL DISCUSSION, SYNTHESIS AND PERSPECTIVES

Prostate cancer (PC) represents a challenging oncological entity, with an increasing incidence with age and a very diverse clinical behavior. After treatment with curative intent, recurrence is common during the first decade. Despite initial response to anti-hormonal therapy, the majority of PC patients will ultimately progress and reach a castration-resistant (CR) state. Recently, several therapeutic options against castration-resistant prostate cancer (CRPC) have emerged, flowing from improved molecular profiling knowledge about the heterogeneous biological behavior of PC. Nevertheless, despite the variety of therapeutic options, proper sequencing (e.g., modality, timing) in individual patients with PC is unclear. To this end, it is essential to develop “instruments” able to identify which phenotype is predominant within PC patients and timely evaluate the response to therapy, thus avoiding futile costly and toxic treatments.

Hybrid imaging techniques (e.g., PET/CT, PET/MRI) play a pivotal role in clinical management of PC, offering both functional and anatomical information. However, the most commonly used oncological PET tracer, [ $^{18}\text{F}$ ]fluorodeoxyglucose ([ $^{18}\text{F}$ ]FDG), shows limited sensitivity for the detection of androgen dependent PC. Alternatively, encouraging results have been reported on the use of radiolabeled choline derivatives as PET tracers for PC [1–5]. Because of its longer half-life (110 versus 20 minutes) and better spatial resolution, [ $^{18}\text{F}$ ]fluoromethylcholine ([ $^{18}\text{F}$ ]FCH) is more convenient for routine clinical use than  $^{11}\text{C}$ -labeled choline, by providing more flexibility concerning imaging protocols and broader availability. Therefore, the aim of this thesis was to technically validate [ $^{18}\text{F}$ ]FCH as initial steps on the road towards imaging guided personalized therapy for metastatic PC.

**Chapter 1** comprises the introduction and outline of this thesis. Some epidemiological aspects and biological characteristics of PC are discussed, followed by an overview of current therapeutic options in metastatic PC. The role of hybrid imaging in PC is outlined, together with the available qualitative and various quantitative approaches. Finally, [ $^{18}\text{F}$ ]FCH is introduced as a promising radioactive tracer, by describing its metabolic pathway and current clinical indications, as well as the potential use for response evaluation and prediction in PC.

Since [ $^{18}\text{F}$ ]FCH is rapidly cleared from the blood pool and, unlike  $^{11}\text{C}$ -choline, is excreted via the kidneys, acquisition protocols have been designed with pelvic imaging prior to bladder filling (i.e., within minutes after injection), followed by a whole body scan after e.g., 30 min – the “dual-phase protocol”. Patterns of tracer uptake as a function

of time help to characterize intraprostatic tracer uptake, as well as sites of suspected haematogeneous metastases. It was suggested that increasing or stable [ $^{18}\text{F}$ ]FCH uptake over time is compatible with malignancy. However, for lymph node assessment this concept has not been validated. Therefore, in **chapter two** we investigated the diagnostic role of dual-phase [ $^{18}\text{F}$ ]FCH PET/CT in a clinical setting. We studied whether time-trends of enhanced radiolabeled choline in lymph nodes (LN) of PC patients can help to discriminate reactive from malignant ones, and whether single time point standardized uptake value (SUV) measurements may also suffice.

25 PC patients with inguinal (presumed benign) and enlarged pelvic LN (presumed malignant) showing enhanced [ $^{18}\text{F}$ ]FCH uptake at dual-phase PET/CT were analyzed. Associations between LN status (benign versus malignant) and  $\text{SUV}_{\text{max}}$  and  $\text{SUV}_{\text{meanA50}}$ , determined at 2 min (early) and 30 min (late) post injection (p.i.), were assessed. We considered two time-trends of [ $^{18}\text{F}$ ]FCH uptake: type A ( $\text{SUV}_{\text{early}} > \text{SUV}_{\text{late}}$ ) and type B ( $\text{SUV}_{\text{late}} \geq \text{SUV}_{\text{early}}$ ). Histopathology and/or follow-up were used to confirm the assumption that LN with type A pattern are benign, and LN with type B pattern malignant.

We identified 54 LN with enhanced [ $^{18}\text{F}$ ]FCH uptake, both at early (2 min p.i.) as well as late (30 min p.i.) time points. Highly significant associations were found between the LN status (inguinal/benign vs. enlarged pelvic/malignant) and the  $\text{SUV}_{\text{max}}$  and  $\text{SUV}_{\text{meanA50}}$  30 min p.i., and their absolute and relative differences ( $p < 0.0001$ ). ROC analyses of uptake trends over time and of SUVs at either time-point showed that the  $\text{SUV}_{\text{max}}$  relative difference was the best predictor of the LN status.

In our referral-based spectrum of patients with enhanced [ $^{18}\text{F}$ ]FCH uptake in pelvic and inguinal lymph nodes, decreasing [ $^{18}\text{F}$ ]FCH uptake over time seems to be a reliable tool to differentiate benign and malignant nodes. Together with similar findings by others to classify radioactive choline positive lesions suspected to represent hematogeneous metastases, our results are relevant for clinical decision making and simplification of diagnostic procedures (e.g., in patients with elevated PSA and positive [ $^{18}\text{F}$ ]FCH PET findings). Moreover, the results underline the relevance of a sequential PET imaging protocol after a single injection of [ $^{18}\text{F}$ ]FCH to account for the time-trend of tracer uptake. Single time-point SUV measurements, 30 min p.i., may be a reasonable alternative for predicting the nodal status, but this remains to be validated in non-enlarged pelvic lymph nodes.

The results presented in our study pertain to the ability of tracer uptake time-trends to classify LN with enhanced [ $^{18}\text{F}$ ]FCH uptake. Reported 'sensitivity' and 'specificity' should not be confused with 'the accuracy' of [ $^{18}\text{F}$ ]FCH PET/CT to diagnose metastatic lymph nodes in prostate cancer. To date, discordant results have been presented in the literature on the usefulness of choline PET/CT in LN staging of newly diagnosed patients with PC. Sensitivity of this technique is influenced by the diameter of metastases and limited by the low PET spatial resolution [6–10]. However, in this setting, other imaging modalities [e.g., transrectal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI)] are less accurate. In case of restaging patients with biochemical relapse, choline PET/CT seems to be a valuable tool, especially in presence of higher prostate-specific antigen (PSA) values and faster PSA kinetics [11, 12]. In this context, the major limitation of the present studies is the histopathological confirmation).

Upon establishing that radioactive choline can play a diagnostic role in clinical practice, in **chapter three** we studied an alternative potential application of [ $^{18}\text{F}$ ]FCH in metastatic PC. Since the actual response to (chemo)therapeutic regimens in individual PC patients is variable, and potentially effective alternative regimens are available, it is important to monitor therapeutic (in)efficacy in time. Presently, this is based on a response metrics construct of PSA measurements, CT scans and bone scintigraphy. The limitations of the current approach are related to the heterogeneity of metastasized PC (i.e., coexistence of androgen sensitive and insensitive components with different impact on e.g., PSA) and to its skeletal predominance (with bone- and CT-scans having difficulties in timely and accurately detecting response). Therefore, there is a clear need for more accurate response monitoring methods.

In-vitro data have shown promising results on the use of PET tracers to monitor anti-androgen treatment (e.g., bicalutamide) or chemotherapy (e.g., docetaxel) in androgen-independent cell lines [13]. There is some recent evidence that [ $^{18}\text{F}$ ]FCH might be also useful as a biomarker of response to anti-androgen therapy in patients with metastatic CRPC [14]. Whether [ $^{18}\text{F}$ ]FCH could also be employed in monitoring treatment response in patients receiving docetaxel and cabazitaxel therapy is unclear. Therefore, we investigated whether accumulation of [ $^{18}\text{F}$ ]FCH, in comparison with [ $^{18}\text{F}$ ]FDG, accurately reflects chemotherapy efficacy at the tumor cell level in PC, both in androgen-dependent and independent cell lines. We analyzed the effects of docetaxel and cabazitaxel on viable tumor cell numbers and tracer uptake in four PC cell lines.

Cellular uptake of [ $^{18}\text{F}$ ]FDG and [ $^{18}\text{F}$ ]FCH was measured using the sulforhodamine B (SRB) assay, cell counting and a colony formation assay (CFA), as metrics for viable tumor cell numbers.

Comparing the reduction in cellular uptake of radioactive [ $^{18}\text{F}$ ]FCH in prostate tumor cells *in vitro* as a function of different dosages of docetaxel and cabazitaxel, with parameters of cell viability, we found that the radiotracer uptake was proportional to the number of residual cells after therapy. These cell numbers correlated well to clonogenic capacity as an additional sign of (reproductive) viability of the cells surviving the treatment. Our *in vitro* data demonstrate that the cellular [ $^{18}\text{F}$ ]FCH uptake fits well with viable tumor cell number after docetaxel and cabazitaxel for all PC cell lines, while [ $^{18}\text{F}$ ]FDG at times overestimated the cell number after drug exposure. This suggests that [ $^{18}\text{F}$ ]FCH is more accurate and therefore more suitable than [ $^{18}\text{F}$ ]FDG to measure the response to docetaxel and cabazitaxel. Therefore, we propose to design clinical studies to validate these findings *in vivo*.

Since experiments *in vitro* suggested radiolabeled choline to be a suitable tracer for response monitoring in PC, in **chapter four** we investigated how [ $^{18}\text{F}$ ]FCH can be applied and reliably measured in a routine clinical setting. As uptake of [ $^{18}\text{F}$ ]FCH should reflect viable tumor tissue, changes over time may serve as a measure of response to therapy. For monitoring response to systemic treatment in metastasized PC, however, accurate quantification is required, preferably with clinically applicable, simple quantitative methods. Such simplifications need to be validated versus the standard kinetic modelling PET methods. Omitting this validation step may seriously confound the clinical biomarker validation process, as has been demonstrated with [ $^{18}\text{F}$ ]FDG and [ $^{18}\text{F}$ ]FLT. Therefore, pharmacokinetic modeling of dynamic PET data in combination with arterial blood sampling was used to determine the appropriate plasma input compartment model for [ $^{18}\text{F}$ ]FCH. In addition, the validity of using an image-derived input function in combination with manual venous blood samples, instead of arterial blood sampling, was investigated, and the validity of using simplified methods for quantification of [ $^{18}\text{F}$ ]FCH was assessed.

Forty-minute dynamic PET/CT scans were acquired after injection of  $204 \pm 9$  MBq [ $^{18}\text{F}$ ]FCH, from eight patients with histologically proven metastasized PC. Plasma input functions were derived using continuous arterial blood sampling (BSIF) as well as using image-derived (IDIF) methods. Manual arterial blood samples were used for

calibration and correction for plasma-to-blood ratio and metabolites. Time activity curves (TAC) were derived from volumes of interest (VOI) in all visually detectable LN metastases. [ $^{18}\text{F}$ ]FCH kinetics were studied by non-linear regression fitting of several single- and two-tissue plasma input models to the TAC. Model selection was based on Akaike information criterion (AIC) and measures of robustness. In addition, the performance of several simplified methods, such as SUV, was assessed.

Best fits were obtained using an irreversible compartment model with blood volume parameter. Correspondence between venous and arterial parent fractions was low, as determined by the intraclass correlation coefficient ( $\text{ICC} = 0.61$ ). Results for IDIF derived from VOI in blood pool structures distant from tissues of high [ $^{18}\text{F}$ ]FCH uptake, yielded good correlation to those for BSIF ( $R^2 = 0.83$ ). SUV showed poor correlation to parameters derived from full quantitative kinetic analysis ( $R^2 < 0.34$ ). In contrast, lesion activity concentration normalized to the integral of the blood activity concentration over time ( $\text{SUV}_{\text{AUC}}$ ) showed good correlation ( $R^2 = 0.92$  for metabolite corrected plasma and  $R^2 = 0.65$  for whole-blood activity concentrations).

We concluded that [ $^{18}\text{F}$ ]FCH uptake should be quantified using full kinetic modeling with  $1\text{T}1\text{k} + V_{\text{b}}$  and metabolite-corrected plasma input function based on arterial blood sampling. Results indicate that SUV cannot be used to estimate [ $^{18}\text{F}$ ]FCH uptake. A clinically feasible alternative could be  $\text{SUV}_{\text{AUC,WB}}$  based on two consecutive PET scans.

Before clinical validation of [ $^{18}\text{F}$ ]FCH PET as a predictive biomarker of response can be initiated, it is essential to know the physiological range of day-to-day variability of the tracer uptake measures. This should help to assess whether observed changes during therapy represent true signal change instead of noise. This “repeatability” of a test is defined as the the measurement precision with conditions that remain unchanged between replicate measurements (repeatability conditions) [15]. Knowledge of these operational characteristics of tests is an essential component of the technical biomarker validation process. Traditionally, changes of tracer uptake have dominated as PET-based response prediction biomarkers. More recently, evidence has emerged that measures of metabolically active tumor volume may also provide relevant information. To this end, in **chapter five**, we assessed the repeatability of various quantitative [ $^{18}\text{F}$ ]FCH parameters, including metabolic tumor volume (MTV) and total lesion choline uptake (TLCU), in PC.

Twelve patients ( $64 \pm 8$  years) with metastasized PC underwent 2 sets of [ $^{18}\text{F}$ ]FCH PET/CT scans, on two successive days. Each set consisted of a 30 minutes dynamic PET/CT scan of the chest, after intravenous administration of 200 MBq [ $^{18}\text{F}$ ]FCH, followed by a whole body (WB) PET/CT at 40 minutes. The dynamic scan was used to derive the area under the curve (AUC) of the blood activity concentration. Lesion uptake was derived from the WB scan using various volumes of interest (VOI): maximum, peak and mean. Each of these parameters was normalized to injected activity/weight, blood AUC and blood concentration itself at 40 minutes, resulting in several SUV,  $\text{SUV}_{\text{AUC}}$  and  $\text{SUV}_{\text{TBR}}$  values. Repeatability of these semi-quantitative parameters, MTV and TLCU, respectively were studied. The level of agreement between test-retest data and reliability was assessed using repeatability coefficients (RC), intraclass correlation coefficients and Bland-Altman plots.

We identified 67 choline avid metastases, 44 bone and 23 lymph node lesions. 12 metastases were located in the chest. In case of  $\text{SUV}_{\text{max}}$ , repeatability coefficients for SUV,  $\text{SUV}_{\text{AUC}}$  and  $\text{SUV}_{\text{TBR}}$  were 26, 31 and 46%, respectively. Similar values were obtained for  $\text{SUV}_{\text{peak}}$  and  $\text{SUV}_{\text{mean}}$ . Repeatability of  $\text{SUV}_{\text{AUC}}$  was comparable with that of standard SUV, for maximum, peak and mean values. Tissue type (e.g., bone versus lymph node) and tumour location did not affect repeatability. Repeatability did not differ between lesions with  $\text{SUV}_{\text{peak}}$  above or below the median value of 8.3 ( $p=0.264$ ). The relative test-retest difference for MTV was 36%. Repeatability of MTV was independent of uptake, tissue type and location. MTV smaller than  $4.2 \text{ cm}^3$  had larger variability than larger volumes (RC 45 versus 28%,  $p=0.048$ ). Repeatability of TLCU and  $\text{TLCU}_{\text{AUC}}$  were comparable (RC 33 versus 31%,  $p=0.954$ ), while  $\text{TLCU}_{\text{TBR}}$  showed larger variance of 51% ( $p<0.001$ ). Repeatability of TLCU was independent of uptake, MTV, tissue type or location.

We concluded that repeatability of  $\text{SUV}_{\text{AUC}}$  was comparable to that of standard SUV, indicating that [ $^{18}\text{F}$ ]FCH PET/CT uptake differences of 30% or more are likely to represent treatment effects. Repeatability of MTV and TLCU, respectively was ~35%. Observed repeatabilities are of the same order of magnitude as those seen for other commonly used radiotracers, such as [ $^{18}\text{F}$ ]FDG and [ $^{18}\text{F}$ ]Fluorotymidine ([ $^{18}\text{F}$ ]FLT).

Since MRI is an important and potentially powerful diagnostic method in PC, perhaps challenging or complementing PET, we questioned if the knowledge acquired about the use of [ $^{18}\text{F}$ ]FCH PET/CT is also applicable to PET/MRI. Therefore, in **chapter six** we



performed a clinical and technical validation of [ $^{18}\text{F}$ ]FCH in PC, by comparing image quality and quantitative accuracy of PET/MRI and PET/CT systems with identical time-of-flight PET gantries, using phantom and clinical studies. The same phantom experiments were performed using both systems, by measuring calibration, uniformity, and SUV recovery. The clinical PET/CT versus PET/MRI comparison was performed using [ $^{18}\text{F}$ ]FCH.

Calibration accuracy and image uniformity were comparable between systems. SUV recovery met EANM/EARL requirements on both scanners. Thirty-four lesions with comparable PET image quality were identified. Lesional SUVmax differences of  $4\pm 26\%$  between PET/MRI and PET/CT data were observed ( $R^2=0.79$ , slope=1.02). In healthy tissues, PET/MRI-derived SUVs were  $16\pm 11\%$  lower than on PET/CT ( $R^2=0.98$ , slope=0.86). Our main conclusion was that PET/MRI and PET/CT showed comparable performance with respect to calibration accuracy, image uniformity, and SUV recovery. [ $^{18}\text{F}$ ]FCH uptake values for both healthy tissues and lesions corresponded reasonably well between magnetic resonance (MR)- and computed tomography (CT)-based systems, but only in regions free of MR-based attenuation artifacts. Nevertheless, the usefulness of PET/MRI remains to be elucidated in clinical, prospective studies with large cohorts of patients.

## SYNTHESIS AND PERSPECTIVES

Taken together, [ $^{18}\text{F}$ ]FCH PET/CT may qualify as a biomarker of response in PC, based on its appropriate lesion to background contrast, its promising in vitro performance in detection of changing viable load during therapy, and the apparent accuracy of generally applicable, simplified quantitative whole body measures. The semi-quantitative methods have repeatability characteristics comparable with those observed with other broadly used tracers (e.g., [ $^{18}\text{F}$ ]FDG, [ $^{18}\text{F}$ ]FLT). As imaging plays an increasing role in the clinical management of PC, we also performed a technical and clinical validation of PET/CT and PET/MRI systems with identical time-of-flight gantries, using [ $^{18}\text{F}$ ]FCH. We found comparable performance between the systems with regard to the technical characteristics and an acceptable clinical correspondence, with limitations mainly due to inherent MR-based attenuation artifacts.

In case of PC, PET/MRI is presumed to be more accurate than PET/CT for primary staging, e.g., by combining high-resolution prostate images (e.g., diffusion weighted imaging (DWI)) and metabolic/molecular imaging ([ $^{18}\text{F}$ ]FCH PET). Comparable PET/CT and PET/MRI performance is expected for LN staging, as nodal disease (especially small < 8 mm metastatic LN) assessment is mainly diagnosed on functional (PET) imaging. For distant metastases, possible advantages of PET/MRI over PET/CT depend on the site of metastatic spread. Potential PET/MRI indications are staging in patients with a positive biopsy, assessment of tumor recurrence after treatment in patients with increasing PSA, and tumor detection in case of increased PSA but negative biopsies. However, MR attenuation correction remains a limiting factor, demanding research for technical improvement [16]. With regard to the whole body PET/MRI systems currently available, there is a clear preference for fully integrated PET/MRI scanners in daily practice. Truly simultaneous PET and MRI acquisitions carry less risk of patient movement since scan time is shorter than with sequential PET/MRI, improving patient throughput at the same time. [16].

Significant progress has recently been made in improving acquisition and interpretation of multiparametric MRI (mpMRI), with an extended role of mpMRI from local staging toward lesion detection and biopsy guidance [17]. Combining both anatomical and functional pulse sequences (i.e., T1-weighted MRI, T2-weighted MRI, DWI or MR spectroscopy), mpMRI shows promising results for biopsy planning and targeting. MpMRI is also useful in the evaluation of extracapsular extension, neurovascular bundle

involvement, seminal vesicle invasion and/or invasion of adjacent structures, such as rectum and urinary bladder, thus preventing futile curative surgery [18]. Developments in the acquisition of mpMRI are ongoing. The DW technique is improved by the use of high b-value DW-MRI. Progress in this field has resulted in implementation of high b-value DW-MRI as a component of the prostate imaging reporting and data system (PIRADS) evaluation criteria [19]. However, an important drawback of mpMRI is the difficulty in detecting small malignant foci in the prostate ( $<0.5\text{ cm}^3$ ) and low-risk Gleason score lesions [20]. Future well-structured studies investigating the best manner to calculate high b-value DW-MRI are needed since functional information hereby provided may guide the optimal therapeutic choice.

Apart from radiolabeled choline, new tracers such as  $^{18}\text{F}$ -16 $\beta$ -18F-fluoro-5 $\alpha$ -dihydrotestosterone ( $^{18}\text{F}$ FDHT), and  $^{68}\text{Ga}$ -PSMA are demonstrating promising results in recurrent and metastatic PC.  $^{18}\text{F}$ FDHT is a biomarker for androgen receptor (AR) expression in human PC, mainly useful in the CRPC state. It has good imaging characteristics, with rapid uptake in malignant cells at metastatic sites expressing AR [21]. Research is ongoing to investigate its role in stratification of patients for systemic therapy and in monitoring treatment effects in patients undergoing novel anti-androgen therapies.

Serial experiments have recently demonstrated the importance of targeting the prostate-specific membrane antigen (PSMA) with either  $^{68}\text{Ga}$ - or  $^{123}\text{I}$ -labelled PSMA inhibitors [22, 23]. PSMA is a membrane-type zinc protease which is negatively regulated by androgen and significantly overexpressed in androgen-independent PC. Increased PSMA expression in PC is associated with higher tumor grade and a high risk of disease progression, as defined by biochemical recurrence after initial curative therapy [24]. Since PSMA elevation is inversely correlated to time to prostate-specific antigen (PSA) progression and disease-free survival, this tracer has the potential to serve as a biomarker for estimating the aggressiveness of PC [24]. Furthermore, PSMA represents an ideal biological target for accurate functional imaging of PC, due to an improved sensitivity to detect small LN-, bone- and liver- metastases [22]. Advantages of  $^{68}\text{Ga}$ -labelled PSMA ligands above choline tracers are twofold: a better signal to background ratio and no cyclotron requirement, since  $^{68}\text{Ga}$  can be extracted from a commercially available  $^{68}\text{Ge}/^{68}\text{Ga}$  radionuclide generator [18]. The role of labeled PSMA in PC is currently under investigation.

Another tracer, a radiolabeled leucine analogue, 1- amino-3-fluorocyclobutane-1-carboxylic acid in the 'anti' configuration ( $[^{18}\text{F}]\text{FACBC}$ ), can be also used to depict amino acid transportation in PC. Since only a small fraction is excreted through the urinary tract after intravenous administration, its imaging characteristics seem to be favorable in the evaluation of prostate cancer [25]. Research data indicate that  $[^{18}\text{F}]\text{FACBC}$  can be successfully used in the assessment of primary and metastatic PC. Preliminary results indicate also that  $[^{18}\text{F}]\text{FACBC}$  may be superior to radiolabeled choline for the identification of disease recurrence in case of biochemical failure [26, 27]. Nevertheless, these findings have to be confirmed in larger, prospective studies.

An important aim of any "diagnosis-treatment" combination is to improve the quality of life of patients with metastatic PC. To validate that  $[^{18}\text{F}]\text{FCH}$  may contribute to this purpose, we suggest the following research, beyond the technical validation described in this thesis. Firstly,  $[^{18}\text{F}]\text{FCH}$  reproducibility studies (measuring test-retest variability in a multicenter setting) are needed to complete the technical validation. Secondly, clinical 'biological validation' studies to define the accuracy and optimal methodology of  $[^{18}\text{F}]\text{FCH}$  to predict clinical response to systemic therapy are needed. Thirdly, comparative  $[^{18}\text{F}]\text{FCH}$  PET/CT and DWI studies may be considered: if DWI and  $[^{18}\text{F}]\text{FCH}$  PET have similar value in overall response prediction, a combination of  $[^{18}\text{F}]\text{FDHT}$  and DWI might help to separate and quantify the androgen-receptor phenotype from the overall tumor load, before and during therapy. Moreover, by opting for active surveillance in case of a coexistent phenotype, these studies will provide the opportunity to timely start effective treatment in case of PC progression. Finally, cost-effectiveness of any diagnosis-therapy combination should be evaluated to support appropriate decision-making by clinicians, patients and health care providers during the management of metastatic prostate cancer.

## REFERENCES

1. Bauman G, Belhocine T, Kovacs M, et al.  $^{18}\text{F}$ -fluorocholine for prostate cancer imaging: a systematic review of the literature. *Prostate Cancer Prostatic Dis.* 2012;15:45–55
2. DeGrado TR, Baldwin SW, Wang S, et al. Synthesis and evaluation of ( $^{18}\text{F}$ )-labeled choline analogs as oncologic PET tracers. *J Nucl Med.* 2001;42:1805–1814
3. Fuccio C, Rubello D, Castellucci P, et al. Choline PET/CT for prostate cancer: main clinical applications. *Eur J Radiol.* 2011;80:e50–e56
4. Picchio M, Briganti A, Fanti S, et al. The role of choline positron emission tomography/computed tomography in the management of patients with prostate-specific antigen progression after radical treatment of prostate cancer. *Eur Urol.* 2011;59:51–60
5. Reske SN, Blumstein NM, Neumaier B, et al. Imaging prostate cancer with  $^{11}\text{C}$ -choline PET/CT. *J Nucl Med.* 2006;47:1249–1254
6. Beheshti M, Imamovic L, Broinger G, et al.  $^{18}\text{F}$  choline PET/CT in the preoperative staging of prostate cancer in patients with intermediate or high risk of extracapsular disease: a prospective study of 130 patients. *Radiology.* 2010; 254: 925–933
7. Poulsen MH, Bouchelouche K, Gerke O, et al. [ $^{18}\text{F}$ ]- fluorocholine positron-emissioncomputed tomography for lymph node staging of patients with prostate cancer: preliminary results of a prospective study. *BJU International.* 2010;106:639–643
8. De Jong IJ, Pruim J, Elsinga PH, Vaalburg W, and Mensink HJ. Preoperative staging of pelvic lymph nodes in prostate cancer by  $^{11}\text{C}$ -choline PET. *J Nucl Med.* 2003;44:331–335
9. Steuber T, Schlömm T, Heinzer H, et al. [ $^{18}\text{F}$ ]-fluoroethylcholine combined in-line PET-CT scan for detection of lymph-node metastasis in high risk prostate cancer patients prior to radical prostatectomy: preliminary results from a prospective histology-based study. *EJC.* 2010;2:449–455
10. Hacker A, Jeschke S, Leeb K, et al. Detection of pelvic lymph node metastases in patients with clinically localized prostate cancer: comparison of [ $^{18}\text{F}$ ]fluorocholine positron emission tomography-computerized tomography and laparoscopic radioisotope guided sentinel lymph node dissection. *J Urol.* 2006;176:2014–2019
11. Breeuwsma AJ, Pruim J, van den Bergh ACM, et al. Detection of local, regional, and distant recurrence in patients with PSA relapse after external-beam radiotherapy using  $^{11}\text{C}$ -choline positron emission tomography. *IJROBPS.* 2010;77:160–164
12. Cimitan M, Bortolus R, Morassut S, et al. [ $^{18}\text{F}$ ]fluorocholine PET/CT imaging for the detection of recurrent prostate cancer at PSA relapse: experience in 100 consecutive patients. *Eur J Nucl Med Mol Imaging.* 2006;33:1387–1398
13. Müller SA, Holzapfel K, Seidl C, Treiber U, Krause B] and Senekowitsch-Schmidtke R. Characterization of choline uptake in prostate cancer cells following bicalutamide and docetaxel treatment. *Eur J Nucl Med Mol Imaging.* 2009;36:1434–1442
14. Caroli P, de Giorgi U, Moretti A, et al. Use of  $^{18}\text{F}$ -Methylcholine PET/CT in early assessment of response to therapy with enzalutamide, in patients with metastatic castration-resistant prostate cancer: results of a pilot study. *J Nucl Med.* 2015;56(Suppl 3):52P
15. Sullivan DC, Obuchowski NA, Kessler LG, et al. Metrology Standards for Quantitative Imaging Biomarkers. *Radiology* 2016;000:1–13
16. Yaqub M, Oprea-Lager DE, Hofman M and Boellaard R. Methodological aspects of PET/MR imaging. *Tijdschr v Nucl Geneesk.* 2013;35:1153–1159
17. Bouchelouche K, Turkbey B and Choyke PL. Advances in imaging modalities in prostate cancer. *Global cancer statistics. Curr opin Oncol.* 2015;27:224–231
18. Picchio M, Mapelli P, Panebianco V, et al. Imaging biomarkers in prostate cancer: role of PET/Ct and MRI. *Eur J Nucl Med Mol Imaging* 2015;42:644–655
19. Barentsz JO, Richenberg J, Clements R, et al. ESUR prostate MR guidelines 2012. *Eur Radiol.* 2012;22:746–757
20. Turkbey B, Mani H, Aras O, et al. Prostate cancer: can multiparametric MR imaging help identify patients who are candidates for active surveillance? *Radiology* 2013;268:144–152
21. Larson SM and Schoder H. New PET tracers for evaluation of solid tumor response to therapy. *QJ Nucl Med Mol imaging.* 2009;53:158–166

22. Afshar-Oromieh A, Zechmann CM, Malcher A, et al. Comparison of PET imaging with a  $^{68}\text{Ga}$ -labelled PSMA ligand and  $^{18}\text{F}$ -choline-based PET/CT for the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging* 2014;41:11–20
23. Barrett JA, Coleman RE, GoldsmithSJ, et al. First-in-man evaluation of 2 high-affinity psma avid small molecules for imaging prostate cancer. *J Nucl Med*. 2013;54:380–387
24. Perner S, Hofer MD, Kim R, et al. Prostate-specific membrane antigen expression as a predictor of prostate cancer progression. *Human Pathology*. 2007;38:696–701
25. Oka S, Hattori R, Kurosaki F, et al. A preliminary study of anti-1-amino-3- $^{18}\text{F}$ -fluorocyclobutyl-1-carboxylic acid for the detection of prostate cancer. *J Nucl Med*. 2007;48:46–55
26. Schuster DM, Taleghani PA, Nieh PT, et al. Characterization of primary prostate carcinoma by anti-1-amino-2- $^{18}\text{F}$ -fluorocyclobutane-1-carboxylic acid (anti-3-  $^{18}\text{F}$ )FACBC uptake. *Am J Nucl Med Mol Imaging*. 2013;3:85–96
27. Nanni C, Schiavina R, Boschi S, et al. Comparison of F-FACBC and  $^{11}\text{C}$ -choline PET/ CT in patients with radically treated prostate cancer and biochemical relapse: preliminary results. *Eur J Nucl Med Mol Imaging*. 2013;40(Suppl 1):S11–17.